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Proinsulin C-peptide – A Consensus Statement

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In recent years the physiological role of the proinsulin C-peptide has received increasing attention, focusing on the potential therapeutic value of C-peptide replacement in preventing and ameliorating type 1 diabetic complications. In order to consolidate these new data and to identify the immediate directions of C-peptide research and its clinical usefulness, an International Symposium was held in Detroit, Michigan, on October 20–21, 2000, under the auspices of the Wayne State University/Morris Hood Jr. Comprehensive Diabetes Center. In this communication, we review the cellular, physiological and clinical effects of C-peptide replacement in animal models and in patients with type 1 diabetes. Finally, recommendations are presented as to the most urgent studies that should be pursued to further establish the biological action of C-peptide and its therapeutic value.

INTRODUCTION

C-peptide fulfills an important function in the biosynthesis of insulin by bringing its A and B chains together. The process of folding and disulfide bond formation, necessary for the generation of bioactive insulin, is thereby facilitated.[1,2] C-peptide is subsequently cleaved from the proinsulin molecule and released to the circulation in amounts equimolar to insulin. Following discovery of the mode of insulin synthesis, several early studies addressed the question of possible insulin-like effects of C-peptide. None were found and it became generally accepted that C-peptide had no other role than its participation in insulin biosynthesis. However, recent reports of cellular binding interactions of C-peptide[3] and the finding of physiological effects in patients with type 1 diabetes, who are C-peptide deficient, have prompted renewed interest in this peptide.[4]

The C-peptide Molecule

The species variability of the C-peptide molecule is greater than for the surrounding A and B chains of insulin, and for many bioactive peptides, but other hormones, like parathyroid hormone and gastrin-releasing peptide, also show considerable inter-species variability. The largely conserved

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mammalian sequence pattern of C-peptides includes four non-consecutive positions with glutamic acid, an interspaced glycine-rich segment, and some further residues. As a result, the C-peptide residue pattern provides a basis for ligand-receptor binding interactions. Their nature is yet unknown, but spacing is obviously important, in a manner like that seen for the major histocompatibility molecules, in which the residue spacing provides sites of defined interactions.[5,6]

Cellular Interactions

Measurements using fluorescence correlation spectroscopy have established the occurrence of C-peptide cell membrane interactions.[3] There is now direct evidence for a stereospecific binding of C-peptide in the nanomolar concentration range to surfaces of several cell types, *e.g.*, renal tubular cells, fibroblasts and endothelial cells. The specificity of the binding is demonstrated by competitive displacement of bound C-peptide by excess concentration of native C-peptide and by the inability of both scrambled C-peptide (the same amino acid residues as C-peptide but randomly assembled) and D-C-peptide to displace binding. The COOH-terminal pentapeptide segment is essential for C-peptide binding and may constitute an active site of the molecule.[3] The binding probably involves a G-protein, since pretreatment of cells with pertussis toxin results in loss of binding. The affinity binding constant for C-peptide is of the order of 3.10^9 M^{-1} and saturation of binding occurs at physiological concentrations (~0.9nM). This finding helps explain why no effects of C-peptide have been observed in healthy subjects or animals–binding site saturation is reached already at physiological C-peptide concentrations. Thus, additional effects cannot be expected from further increases in C-peptide concentrations. C-peptide binding is not displaced by molar excess of insulin, proinsulin, IGF-I, IGF-II, or NPY, nor is insulin bound to cell membranes displaced by C-peptide. However, it cannot be excluded that C-peptide binds to *e.g.*, the insulin receptor, but at a different site than that for insulin itself.

Indeed, recent studies suggest a possible interaction between C-peptide and insulin. Data from studies in isolated human skeletal muscle strips indicate that C-peptide stimulates glucose transport, but *via* a mechanism that is independent of the insulin receptor and of tyrosine kinase activation.[7] On the other hand, in rat L6 myoblasts, physiological concentrations of C-peptide has been shown to autophosphorylate the insulin receptor, stimulate tyrosine kinase, IRS-1 tyrosine phosphorylation, P13 kinase activity, MAPK phosphorylation, p 90 Rsk and GSK-3 phosphorylation.[8] These effects result in glycogen synthesis and amino acid uptake. Similar effects cannot be demonstrated by scrambled C-peptide. The data suggest the possibility that the insulin signaling pathway may be activated by both C-peptide and insulin.[8]

A short term effect of C-peptide on glucose utilization has been demonstrated in type 1 patients with the euglycemic clamp technique. These studies demonstrate a 25% increase when C-peptide was acutely raised to physiological levels, whereas further increases had no additional effect.[9] These findings correlate with measurements of increased glucose uptake by resting and exercising forearm muscle during C-peptide infusion.[10] and increased glucose uptake by muscle-strips from diabetic subjects.[11] However, in patients receiving C-peptide plus insulin for 3 months, blood glucose levels, indices of metabolic control, and insulin doses, were all unchanged as compared to non C-peptide treated controls.[7]

The effects of C-peptide concerning two of the characteristic abnormalities in type 1 diabetes, *i.e.*, impaired Na^+, K^+-ATPase activity and reduced endothelial function are probably more important than the C-peptide influence on glucose utilization. A first outline of an intracellular signal transduction pattern now emerges. Exposure of cells to C-peptide in physiological concentrations results in

activation of a G-protein, a rise in intracellular Ca^{2+}-concentration from extracellular sources and subsequent activation of Ca^{2+}-dependent protein phosphatase IIB, leading to conversion of inactive phosphorylated Na^+, K^+-ATPase to its active dephosphorylated form.[12] The COOH-terminal pentapeptide is as effective as the intact C-peptide molecule in stimulating Na^+, K^+-ATPase activity.[13] In addition, in endothelial cells the rise in Ca^{2+}-concentration is accompanied by augmented activity of endothelial nitric oxide synthase (eNOS).[14] Furthermore, several findings suggest that C-peptide may interact with other hormones and growth factors. The effect of C-peptide on Na^+,K^+-ATPase is potentiated by the presence of sub-threshold concentrations of neuropeptide Y.[12]

Physiological Effects

C-peptide has been found to elicit concentration-dependent stimulation of Na^+, K^+-ATPase activity in a variety of tissues including renal tubular cells,[12] rat sciatic nerve,[15,16] pancreatic islets,[17] granulation tissue[16] and red blood cells.[14,18] C-peptide exerts an ameliorating effect on the impaired deformability of red blood cells from type 1 diabetic patients, which is probably mediated *via* its effects on Na^+, K^+-ATPase. Further support for the C-peptide effects on Na^+, K^+-ATPase is provided by its effect on rat sciatic nerve Na^+, K^+-ATPase in type 1 diabetic BB/Wor-rats treated with C-peptide for 8 months.[15,19] This effect is further substantiated by partial correction of the Na^+, K^+-ATPase associated defect in nerve conduction velocity and paranodal swelling, secondary to axonal Na^+ accumulation.[19] Furthermore, in the C-peptide deficient diabetic BB/Wor-rat model the expression of both the insulin receptor and the IGF-I receptor mRNA and protein in peripheral nerve and brain tissue were normalized by C-peptide replacement,[20,21] and the diabetes-induced hippocampal apoptosis was prevented by C-peptide replacement.[21]

In type 1 diabetic patients, autonomic nerve function as measured by heart rate variability during deep breathing improves after intravenous C-peptide infusion for 3h,[22] and in patients who receive C-peptide for 3 months.[7] A subgroup of the latter patients with signs of sensory neuropathy exhibits improved temperature threshold discrimination after C-peptide replacement for 3 months.[7] None of these effects are seen in patients who receive insulin therapy alone. There is no immediate explanation for the beneficial effects of C-peptide on nerve function and structure. It may be related to a stimulation of nerve Na^+, K^+-ATPase activity, resulting in improved electrolyte balance and enzyme state. Alternatively, the effects may be a consequence of improved neural blood flow. Several studies demonstrate an effect of C-peptide on NO-release. It stimulates eNOS, causing release of NO from bovine aortic endothelial cells in a concentration-dependent manner, an effect that is abolished by NO-synthase inhibitors.[23] This is in keeping with the finding that C-peptide induces an increase in forearm blood flow in type 1 diabetic patients, which is blocked by a NO-synthase blocker.[24] It is also consistent with the demonstration of a C-peptide concentration-dependent dilatation of rat skeletal muscle arterioles in the presence of insulin.[25]

C-peptide replacement in streptozotocin diabetic rats extending from 120 min to 14 days results in diminished glomerular hyperfiltration, increased functional reserve and reduced urinary albumin extraction.[26,27] In addition, the renal weight tends to be lower and the glomerular volume was significantly smaller in C-peptide treated animals then in non-treated controls.[27] These data have been confirmed in clinical studies in patients with type 1 diabetes. Thus, C-peptide replacement during 3h and during 1 month in young patients with early signs of diabetic nephropathy, *i.e.*, glomerular hyperfiltration but absence of manifest microalbuminuria, was accompanied by reduced glomerular hyperfiltration and decreased filtration

fraction.[9,28] Furthermore, in a double-blind, randomized, cross-over study, C-peptide replacement for 3 months in patients with microalbuminuria resulted in a 40% reduction in urinary albumin excretion.[7] It may be hypothesized that C-peptide has the potential, *via* stimulation of Na^+, K^+-ATPase, to influence membrane permeability of the nephron and to normalize regional blood flow, resulting of improvements in renal function in the diabetic state.

Little information is available regarding the possible influence of C-peptide on retinopathy in type 1 diabetes. However, one study demonstrates diminished leakage of fluorescein across the blood-retinal barrier after 4 weeks of C-peptide replacement in young type 1 patients,[24] an effect possibly related to the ability of C-peptide to stimulate blood flow and increase capillary recruitment.[29]

Summary

Experimental and clinical data now convincingly demonstrate that C-peptide exerts biological effects of its own. The effects are concentration-dependent, in the low physiological range, and most likely mediated *via* stimulation of Na^+, K^+-ATPase and eNOS activities. Animal and human data indicate that C-peptide has a preventive and ameliorating effect on the chronic complications in type 1 diabetes particularly as regards diabetic neuropathy and nephropathy. However, its mode of receptor interaction, signaling pathways and potential interaction with insulin or other hormones are still incompletely understood. Therefore, based on the deliberations at this consensus meeting, the C-peptide study group agreed on the following recommendations:

Recommendations

1. Efforts should be directed to identify and characterize the C-peptide receptor and to explore as to whether C-peptide interacts with other receptors.
2. Further work will be needed to identify signaling pathways utilized by C-peptide.
3. Interactions with other hormones, growth factors and transcription factors need to be explored.
4. Elucidation of the effect of C-peptide on blood flow in the target tissues of diabetic complications should be persued.
5. The anti-apoptotic effect of C-peptide should be examined in different tissues and cell systems.
6. Examination of the effects of C-peptide on free radical formation and scavenging in tissues of diabetic complications should be performed
7. Clinical trials should be conducted with respect to diabetic neuropathy and nephropathy.
8. The effect of C-peptide replacement on the development of diabetic retinopathy should be examined in appropriate animal models.
9. Appropriate delivery systems should be designed for convenient and continuous administration of C-peptide.

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